Please insert the following section heading immediately before paragraph [0001] of the specification:

CROSS REFERENCE TO RELATED APPLICATIONS

Please replace the original paragraphs of the specification having the identifying numbers set out below with the following clean replacement paragraphs, and insert paragraph [0001.1] immediately after paragraph [0001]:

[0001]   This application claims priority to U.S. Provisional Application No. 60/268,401 filed February 12, 2001 and U.S. Provisional Application No. 60/275,022 filed March 12, 2001, the contents of which are incorporated herein by reference.

[0001.1]   The present invention is related to the systems and methods for detecting and identifying molecular structures and binding events, and more particularly to systems and methods for monitoring the change in the permittivity of the test sample in which the molecular structure or binding event resides to detect and identify molecular structures and binding events.

[0026]   Fig. 1 illustrates a permittivity test set configured to determine the permittivity of the test sample in accordance with one embodiment of the present invention. The test system includes a computer 105, a signal analyzer 110, and a detector assembly 120. Computer 105 controls the settings and operation of signal analyzer 110 via a command bus 107 (a general purpose instrument bus in one embodiment). Responsive to the computer's instructions, signal analyzer 110 transmits an incident signal 111 along a signal path 112 (typically a coaxial cable) to the detector assembly 120. Within the detector assembly 120, a detector 122 is positioned proximate to the test sample 130, such that the detector 122 is electromagnetically coupled (either directly or indirectly, as defined above) to the test sample 130. In a specific embodiment, the test sample 130 is an aqueous environment which may contain molecular structures 132 and/or or binding events 134 as defined above.

[0027]   As the incident signal 111 illuminates the test sample 130, the dielectric properties of the test sample 130 modulate the incident signal 111. At least a portion of the

2

modulated incident signal is reflected back toward and is recovered by the detector element 122. The incident and response signals 111 and 113 are subsequently analyzed to calculate the measured permittivity of the test sample 130.

[0030]   Fig. 2B illustrates the internal architecture of the computer system 105 of Fig. 2A. The computer system includes monitor 214 which optionally is interactive with the I/O controller 224. Computer system 210 further includes subsystems such as system memory 226, central processor 228, speaker 230, removable disk 232, keyboard 234, fixed disk 236, and network interface 238. Other computer systems suitable for use with the described method can include additional or fewer subsystems. For example, another computer system could include more than one processor 228 (*i.e.*, a multi-processor system) for processing the digital data. Arrows such as 240 represent the system bus architecture of computer system 210. However, these arrows 240 are illustrative of any interconnection scheme serving to link the subsystems. For example, a local bus could be utilized to connect the central processor 228 to the system memory 226. Computer system 210 shown in Fig. 2 is but an example of a computer system suitable for use with the present invention. Other configurations of subsystems suitable for use with the present invention will be readily apparent to of skill in the art.

[0032]   As illustrated in Fig. 1, two types of coupling elements are used in the preferred embodiment of the invention: a dielectric measurement probe and a detector assembly. The dielectric measurement probe (not shown) is used during the calibration process, further described below. In a specific embodiment, the dielectric measurement probe is model no. HP 85070C dielectric measurement probe available from Agilent Technologies, Inc. (Palo Alto, CA). The detector assembly 122 is operable to measure the permittivity of the test sample, the process of which is also further explained below.

[0033]   Fig. 3A illustrates one embodiment of a resonant detector 330 used to determine the permittivity of a test sample in accordance with the present invention. A specific embodiment of the detector is described in greater detail in applicant's commonly-owned, co-pending patent application no. 09/687,456 entitled: "System and Method for Detecting and Identifying Molecular Events in a Test Sample."

[0039]   Fig. 3B illustrates an exemplary return loss response (referred to as a S_{11}) of the type that can be obtained using a resonant coaxial fixture. The response is characterized by an

amplitude response (y-axis) extending over one or more frequencies (x-axis). As illustrated, the response exhibits a minimum amplitude at a frequency f_{res} typically referred to as the resonant frequency of the resonant probe. At this frequency, signal power will be substantially retained within the resonant probe. This is the frequency at which the probe is most sensitive since little power is dissipated within the probe itself. A parameter referred to as the "Quality" or "Q"-factor is used to measure how well the probe (or any resonant structure) retains signal power at its resonant frequency. Generally, the Q-factor is a ratio of the energy stored versus the energy dissipated at the resonant frequency f_{res}. Mathematically, the Q-factor can be expressed as:

(2) $Q = f_{res}/\Delta f_{3dB}$

where: f_{res} is the resonant frequency at which the S11 amplitude is minimum; and

Δf_{3dB} is the -3dB or half power bandwidth of the resonant detector above and below f_{res}

[0046]   Fig. 3C illustrates one embodiment of a non-resonant detector, realized as an open-ended coaxial probe 350 (hereinafter referred to as "non-resonant probe"). The non-resonant probe 350 includes a section of open-ended coaxial line 351, an interaction fixture base 353, an interaction substrate 355, a fluid interface 357 having one or more fluid tubes 359 extending therefrom. In a specific embodiment, the probe 350 is coupled to a network analyzer or similar test equipment capable of measuring incident and reflected signal properties.

[0051]   The second dielectric plate 379 includes a channel 377 formed on the top surface and metallization deposited on the bottom surface. The channel 377 is aligned with channel 372 to form a cavity within which the flow tube 375 extends. The metallization 378 deposited on the bottom surface functions as the ground plane of the microstrip detector and will typically consist of a highly conductive material such as those described above. In an alternative embodiment, ground plane metallization may be deposited on the top surface of the top dielectric plate 374 to form a coplanar waveguide structure. Those of skill in the art of high frequency circuit design will appreciate that other configurations are also possible. In the illustrated

embodiment, .002" of copper is used as the bottom surface metallization to provide the detector's ground plane.

[0052]   As shown in Fig. 3D, channels 372 and 377 are aligned to form a cavity which retains the flow tube 375 in a substantially vertically aligned position between the transmission line 371 and the ground plane 378. The flow tube is held between the transmission line 371 and the ground plane 378 along a longitudinally distance 373 referred to as the detection region. This configuration results in the passage of a significant number of field lines emanating from the transmission line through the flow tube (and accordingly, the test sample) before terminating on the ground plane 378. As discussed previously in this and the related applications, the dielectric properties of the sample flowing through the detection region will modulate the signal propagating along the transmission line 371 (*i.e.*, by altering the field lines setup between the transmission line 371 and ground plane 378), thereby providing a means to detect and identify analytes or binding events occurring in the test sample.

[0053]   In one embodiment, the microstrip detector includes connectors (not shown) connected to the transmission line 371 and ground plane 378 on the near and far sides. Suitable connectors are selected based upon the desired test frequency, N-type connectors being suitable for low frequency tests (< 100 MHz), and SMA, K-type, 3.5 mm or 2.4 mm connectors being more suitable for higher frequency tests. Connection by other means, such as coplanar waveguide probes, may also be used in alternative embodiments.

[0055]   The flow tube 375 supplies the test sample through the detection region along 373 between the transmission line 371 and ground plane 378. In the preferred embodiment, the flow tube 375 is constructed from a material having a low loss tangent and a smooth, resilient surface morphology which inhibits analyte formation along the inner surface. A PTFE tube having an ID of .015" and OD of .030" is used in the illustrated embodiment, although other materials and/or sizes may be used as well. For example, materials such as ETFE or other materials described in this and the related cases may be used in alternative embodiments. Further, the flow tube 375 may consist of a microfluidic capillary such as those discussed in applicant's commonly owned, co-pending U.S. Patent Application serial no. 09/687,456, entitled "System and Method For Detecting Molecular Events in a Test Sample." Applicant's commonly-owned, concurrently filed patent application entitled "Bioassay Device for Detecting

Molecular Structures and Binding Events," (Atty Dkt No. 15.0 US) further describes other non-resonant bioassay detectors, each of which may be similarly used in the aforementioned process.

[0057]　Fig. 4A illustrates one embodiment of the detector assembly 120 used to determine the permittivity of a test sample in accordance with the present invention. In this embodiment, the detector assembly 120 includes a fluid transport system 450 integrated with a detector 330. Embodiments of the detector assembly 120 is described in greater detail in applicant's commonly owned, co-pending patent application no. 09/678,456 entitled "System and Method for Detecting and Identifying Molecular Events in a Test Sample."

[0058]　The sample transport system 450 includes a fluid channel 451, with a entry end 452 and an exit end 454. Motion of the test sample through the channel 451 is controlled by a fluid controller 456, which acts to move the test sample through the channel at times and under conditions selected by the user. Optionally, reservoir 458 can include a second analyte or test sample that can be mixed with the test sample stored in reservoir 457 as they are being introduced to the fluid channel 451. The ability to mix two test samples in close proximity to the detector makes it easy for the kinetics of binding events to be determined from this type of data. The fluid controller 456 can move the test sample in one direction, in forward and reverse directions, or pause the test sample for a predetermined duration, for instance, over the detection region in order to improve sensitivity.

[0059]　The detector assembly 330 includes probe head 330a and connecting end 330b. The probe head 330a is positioned proximate to the detection region 455 of the fluid channel 450 and is operable to electromagnetically couple (directly or indirectly, as defined above) the incident test signal to the test sample flowing through the detection region 455. The test sample modulates the incident signal, a portion of which is reflected to the probe head 330a. The reflected modulated signal is subsequently recovered by the probe head 330a. The connecting end 330b is electrically connected (directly or via intervening circuitry) to the signal analyzer 110 (shown in Fig. 4B). In a specific embodiment in which the detector is a coaxial-type structure, the connecting end 330b can be a coaxial cable which extends from the signal analyzer, a compatible coaxial type connector such as a SMA-type connector, or other connector type familiar to those skilled in the art of high frequency measurement. In alternative embodiments of the invention in which a different type of probe architecture is used (*i.e.*,

coplanar waveguide, microstrip, etc.), the connection port can comprise a compatible connection to provide signal communication to the permittivity test set.

[0060]   Fig. 4B illustrates a second embodiment of the detector assembly 120. In this embodiment, the detector assembly 120 includes a length of RF permeable tubing 370, one example being PTFE type-tube available from Cole-Parmer Instrument Company (Vernon Hills, IL). The tubing 370 transports the test sample to the detection region 371 illuminated by the detector 330. A cover piece 372, which is preferably constructed from a conductive material, includes a grooved portion through which tubing 370 extends.

[0061]   In the illustrated embodiments of Fig. 4A and 4B, the probe head 330a is indirectly coupled (as defined above) to the test sample by closely positioning the probe head proximate to the test sample. The intervening material(s) that physically separates the probe head 330a from the test sample can include solid phase materials, such as PTFE, alumina, glass, sapphire, diamond, Lexan®, polyimide, or other dielectric materials used in the area of high frequency circuit design; materials used in the fabrication of microfluidic devices or semiconductor processing; or other known materials which exhibit a relatively high degree of signal transparency at the desired frequency of operation. In a specific embodiment, the intervening material can be an electrically insulating material, some examples of which are described above. In embodiments in which the outer wall of a tube is the intervening material, the material may also be visually translucent or transparent to permit visual inspection of the sample as it moves through the tube. In specific embodiments, the tube may be made from fluoropolymers, such as PFA (perfluoro alkoxy alkane), PTFE (poly-tetra-fluoro-ethylene), FEP (fluorinated ethylene propylene), or ETFE (ethylene-tetrafluroethylene, copolymer), to name a few. The flow cell (not shown) may be constructed from a variety of materials such as (poly) methyl methacrylate - PMMA – acrylic, polycarbonate (known as Lexan®), or polyetherimide (known as Ultem®), as well as others.

[0063]   The thickness and dielectric properties of the intervening materials can vary depending upon the type of fluidic system implemented and detector used. For instance, in systems in which the separation distance is great, a low loss, high dielectric material is preferred to provide maximum coupling between the test sample and the probe 330. In systems in which the separation distance is relatively short, materials of higher loss and lower dielectric constant

can be tolerated. In a specific embodiment in which the channel 451 is PTFE tube having

dimensions of 0.031 inch I.D., 0.063 inch O.D., wall thickness 0.016 inch, and a dielectric

constant of approximately 2, the separation distance is approximately the tube's wall thickness,

about 0.016 inch. In other detector assemblies, separation distances can be on the order of 10^{-1}

m, 10^{-2} m, 10^{-3}m, 10^{-4}m, 10^{-5}m, or 10^{-6}m, and can be much smaller, e.g., on the order of 10^{-9}m in

some cases (such as in a channel etched into the surface of a substrate and having a metallic

signal path element with a thin polymer layer on the test sample side acting as the fourth side of

the channel). Decreasing the separation distance or increasing the detection area 455, the sample

volume, or analyte concentration will operate to increase detection sensitivity. The separation

material, as illustrated above, can a solid phase material, or alternatively (or in addition) consist

of a liquid or gaseous phase material or a combination thereof.

[0064] In an alternative embodiment, the probe head 330a and test sample may be

directly coupled (as defined above), in which case the test sample comes into direct contact with

the probe head 330a. In this embodiment, measurement sensitivity is increased as the signal loss

contributed by the intervening material is not present. This embodiment may be realized in a

variety of ways, for instance in the probe of Fig. 3A by extending the center conductor 335 such

that it contacts the test sample moving through the detector region 455 of the fluid channel 451

of Fig. 4A. In such an embodiment, the channel substrate (the material on which the fluid

channel 451 is formed) may include a cavity within the detector region 351 for receiving the

center conductor 335. The dielectric properties of the channel substrate may be used and the

outer conductor of the detector extended to maintain the characteristic impedance of the detector

(typically 50 ohms). Alternative realizations in which the test sample contacts the probe head

330a in the illustrated and alternative embodiments will be readily apparent to those skilled in

the art.

[0076] In a specific embodiment, the first calibration sample is phosphate buffer

solution (referred to as "PBS"). In an alternative embodiment, other materials such as DMSO,

de-ionized water may be used as the calibration solution. In a specific embodiment, the signal

analyzer is a vector network analyzer model no. HP 8722, and the measurement probe is the

aforementioned model no. HP 85070C dielectric measurement probe. The dielectric probe